LOW-LEVEL CYCLO-SARIN (GF) VAPOR EXPOSURE IN RATS: EFFECT OF EXPOSURE CONCENTRATION AND DURATION ON PUPIL SIZE

C.E. Whalley, B.J. Benton, J.H. Manthei, R.A. Way, E.M. Jakubowski, Jr., D.C. Burnett, B.I. Gaviola, J.S. Forster, R.B. Crosier, D.R. Sommerville, W. Muse, R.J. Mioduszewski and S.A. Thomson
U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD

J.A. Scotto, J. Edwards, D.B. Miller, C.L. Crouse, and K.L. Matson
Geo-Centers Inc., Gunpowder, MD

ABSTRACT

The probability of cyclo-sarin (GF) vapor-induced miosis (defined as a post-exposure pupil diameter 50% or less of the pre-exposure pupil diameter) was estimated in rats exposed to various combinations of exposure concentration and duration. Groups of male and female Sprague-Dawley rats were exposed to GF vapor for a single duration (10, 60 or 240 minutes) in a whole-body dynamic chamber. Pupil diameter was measured by an infrared camera technique. For the six combinations of gender and exposure duration, the effective concentration for miosis in 50% of the exposed population (EC_{50}) and the common probit slope were determined. Contrary to Haber's rule, ECt_{50} values increased with exposure duration (i.e., the Ct for 50% of the exposed population to show miosis was not constant over time). Female rats were more sensitive to GF vapor toxicity than male rats. Miosis was the only clinical sign noted following GF vapor exposure. Possible depression of blood esterase (acetylcholinesterase, butyrylcholinesterase and carboxylesterase) activities due to low-level range of GF vapor concentrations was also investigated. GF was regenerated from blood samples of vapor-exposed rats by the addition of fluoride ion at pH 4 and the samples were analyzed by GC-FPD and GC-MS. Levels of regenerated GF in the red blood cell (RBC) fraction of the samples were five to 40 times lower than in plasma. All controls were negative for regenerated GF.

INTRODUCTION

Acute low-level exposure to Cyclo-Sarin (GF) vapor results in both systemic and local toxic effects, which are mediated primarily via inhalation and ocular routes, respectively. The first eye sign to appear following whole-body exposure to low dose nerve agent vapor is miosis, which may be accompanied by a sensation of dimness of vision. With increasing doses, this may be accompanied by ciliary spasm, headache, and eye pain (Sidell, 1992). In estimating the biological impact of GF vapor exposure on the eye, it is necessary to quantitatively relate the probability of eye responses, such as miosis, to exposure parameters. At minimum, these exposure parameters include atmospheric concentration (C) and exposure duration or time (t). The difficulty in using Ct to compare data from different studies is the traditional assumption that integration of vapor concentration over time (Ct or dosage) for any biological effect is constant (Haber's rule; Haber, 1924). Previously, it was reported that the relationship between exposure concentration-time and lethal response in rats exposed to GB (Sarin)

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 JUL 2003	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Low-Level Cyclo-Sarin (Gf) Vapor Exposure In Rats: Effect Of Exposure Concentration And Duration On Pupil Size	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001523., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**7**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

vapor (Mioduszewski et al., 2001) could not be adequately described by Haber's rule. The objective of the present study was two-fold: a) to determine the EC_{50} for GF vapor-induced miosis and associated probit slope in the rat and b) to model the relationship between GF vapor exposure concentration (C), duration of exposure (t), and the probability of miosis. This study examined the relationship between exposure concentration and miosis in rats exposed to GF vapor for 10, 60 or 240 minutes.

OBJECTIVES

The objective of this study was to determine the exposure conditions for GF vapor-induced miosis in rats: a) EC_{50} (miosis) for 10, 60 and 240 min exposures and b) examine potential male vs. female differences. Is Ct constant over time for miosis? If not, what model best describes the relative influence of exposure concentration (C) and duration (t) on the probability of miosis?

METHODS

Cyclo-Sarin (GF) vapor was generated as described below. The saturator cell vapor generator, a multi-pass glass cell containing a ceramic cylinder saturated with GF (100.5 ± 0.7% pure by NMR) (Fig. 1, left), was immersed in a constant temperature bath. The carrier gas (nitrogen) passed through the cell and mixed with environmentally controlled air into a 750 L dynamic flow chamber (Fig. 1, right). The nitrogen gas flow rate, the saturator cell temperature and the dilution airflow rate controlled the vapor concentration in the exposure chamber.

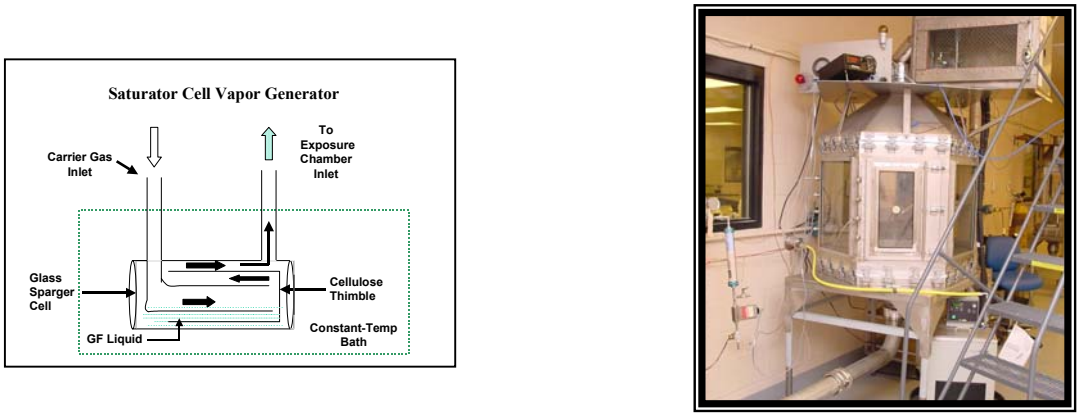

Figure 1. Saturator cell vapor generator (left) and dynamic flow 750 L chamber used for whole body vapor exposure (right).

Vapor sampling/analysis was performed using Solid Sorbent Tubes (Tenax-TA) - Dynatherm + GC/FID. A phosphorus monitor (HYFED-PH 262) provided continuous chart record for rise, equilibrium and decay of chamber vapor.

The following experimental design was used: a) Exposure times: 10, 60, and 240 min; b) GF vapor dose-response using four GF concentrations and an air control per exposure time; and c) at least 20 (10 females + 10 males) adult Sprague-Dawley rats per concentration.

The miosis endpoint measured before/after GF vapor or air exposure was pupil size at -48 hr, -24 hr, -1 hr, +30 min, +1 day, +2 days, and +7 days) (Note: Miosis was defined as at least 50% shrinkage of pre-exposure pupil size). An Infrared Video Imaging System/Pupillometer was used to measure pupil

size under low light conditions (~ 1 foot candle). This system was an IR capable Sony CCD black and white video camera with a 75 mm/F2.7 lens. The attached computer system possessed a National Instruments image acquisition card and custom software written in National Instruments LabView and Imaq code**.**

The following blood esterase activities were measured at -24 hr, +60 min and +7 days: Acetyl cholinesterase (AChE), Butyrylcholinesterase (BuChE), and Carboxylesterase (CaE).

GF regeneration from blood at -24 h, +60 min and +7 days was performed. Fluoride ion at pH 4 was added to plasma and red blood cells (RBC) and samples were analyzed by GC-FPD and GC-MS.

Data analysis was as follows. Probit analysis for miosis (with EC_{50}, common slope and 95% fiducial limits) was generated with Minitab (Version 13). A male versus female probit analysis was performed. A separate pupil size versus concentration curve for each combination of gender and duration was fit by non-linear modeling. Modeling was as follows: a) Relative effects of exposure concentration and duration on probability of miosis and b) Multi-factor probit analysis (Minitab, V. 13) of combined data.

RESULTS

The EC_{50}, ECt_{50}, slope and fiducial intervals for miosis in rats exposed to GF vapor for 10, 60 and 240 min are presented in Table 1 below.

Table 1. EC_{50}, ECt_{50}, Slope and Fiducial Intervals for Miosis in Rats Exposed to GF Vapor for 10, 60 and 240 min.

Exposure Duration (min)	Slope	STD ERR Slope	EC_{50} (mg/m^3) Female	95% F.I. Female	EC_{50} (mg/m^3) Male	95% F.I. Male
10	4.09	0.50	0.060	0.045-0.079	0.186	0.141-0.250
60	4.09	0.50	0.025	0.019-0.033	0.044	0.032-0.065
240	4.09	0.50	0.017	0.013-0.023	0.030	0.022-0.041

Exposure Duration (min)		ECt_{50} ($mg\text{-}min/m^3$) Female	95% F.I. Female	ECt_{50} ($mg\text{-}min/m^3$) Male	95% F.I. Male
10		0.60	0.45-0.79	1.86	1.41-2.50
60		1.48	1.14-1.99	2.66	1.91-3.87
240		4.17	3.45-5.50	7.17	5.30-9.82

Toxic Load Model For Miosis in Rats exposed to GF vapor: $C^n t = k$ or $Normit = b_0 + b_1 Log(C) + b_2 Log(t)$. The toxic load exponent (n) is 2.05, with approximate standard error 0.21 (Fig. 2). $Normit = 1.875 - 0.645 \cdot Sex + 3.649 \cdot Log(C) + 1.778 \cdot Log(t)$. Sex was coded -1 for female rats and +1 for male rats.

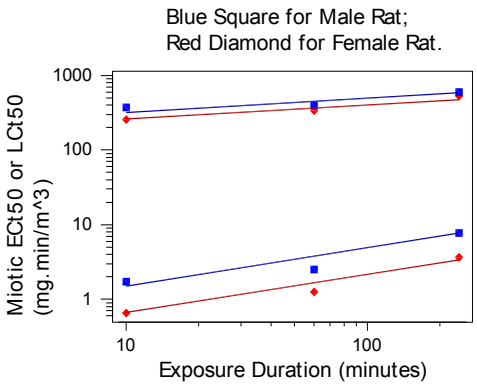

Figure 2. Comparison of GF miosis and lethality: toxic load models. Lower lines represent miosis; upper lines represent lethality.

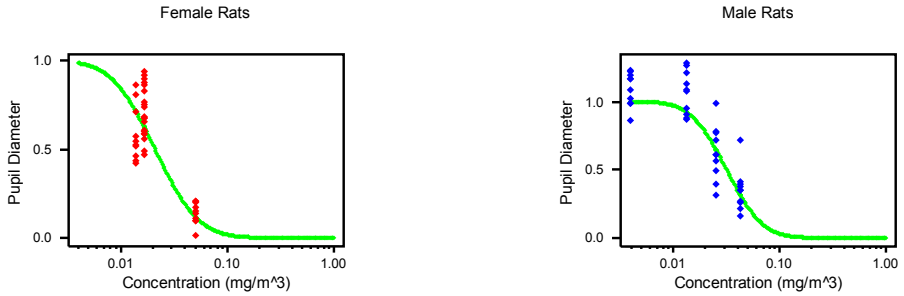

Figure 3. Effects of 10 min GF vapor exposure (various concentrations) on pupil diameter of female (left) and male rats (right).

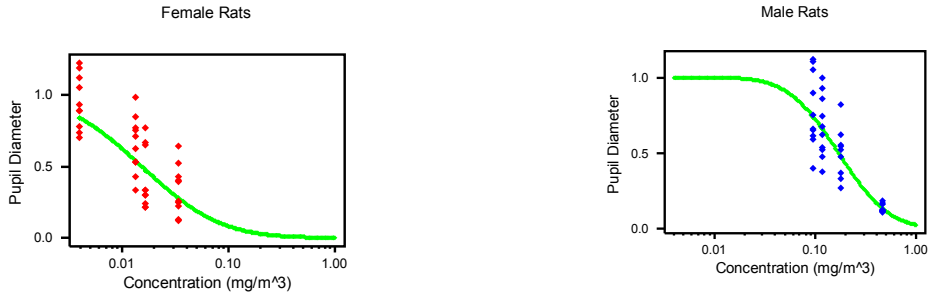

Figure 4. Effects of 60 min GF vapor exposure (various concentrations) on pupil diameter of female (left) and male (right) rats.

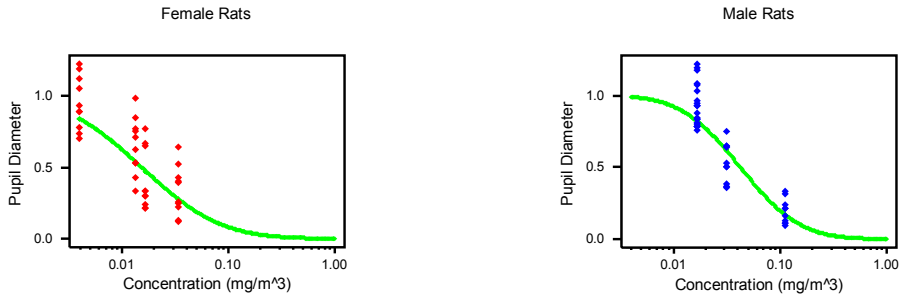

Figure 5. Effects of 240 min GF vapor exposure (various concentrations) on pupil diameter of female (left) and male (right) rats.

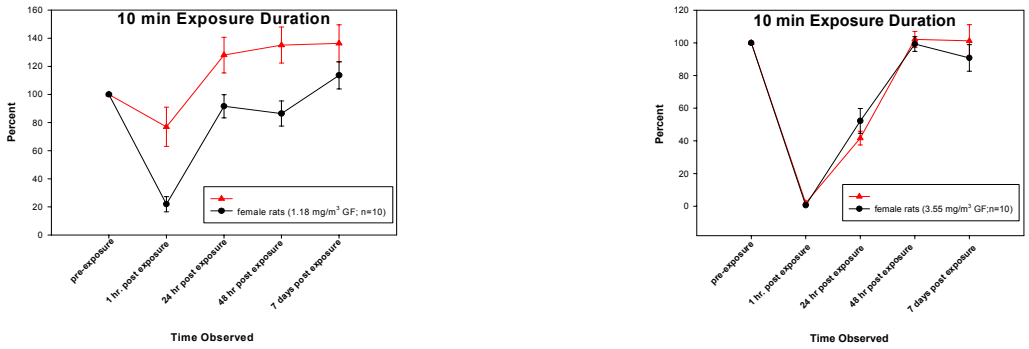

Figure 6. Time course of 10 min GF vapor exposure at different concentrations on pupil diameter in male and female rats.

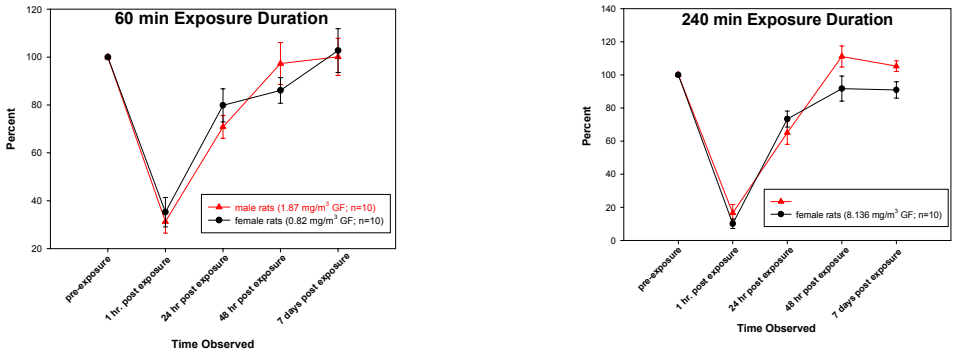

Figure 7. Time course of 60 and 240 min GF vapor exposure on pupil diameter in male and female rats.

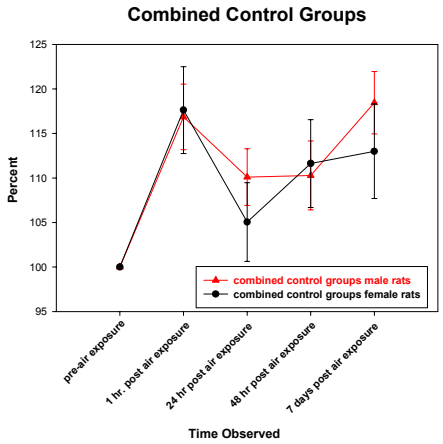

Figure 8. Effects of air exposure on pupil diameter of male and female rats.

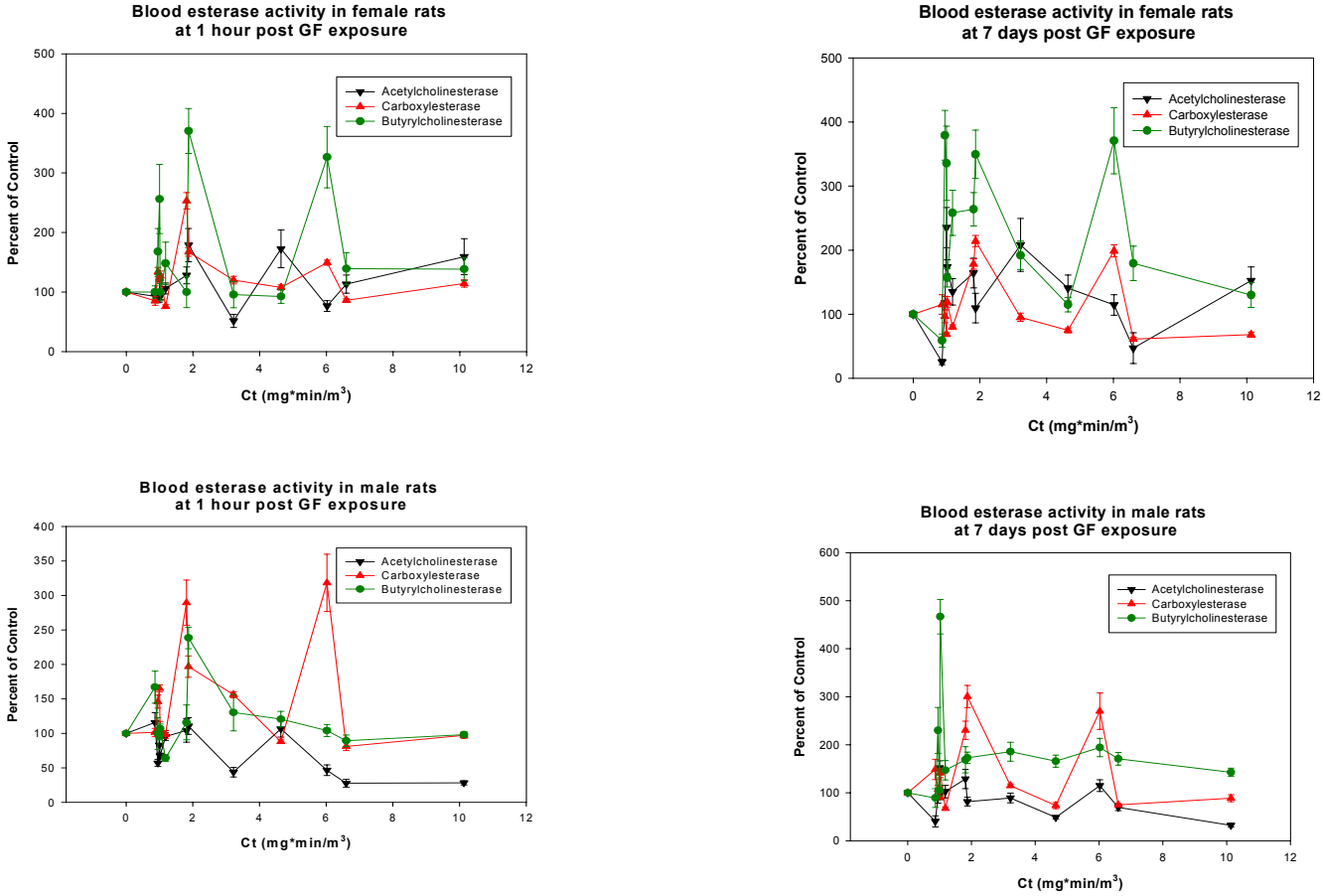

Figure 9. Effects of GF vapor exposure on rat blood esterase activity.

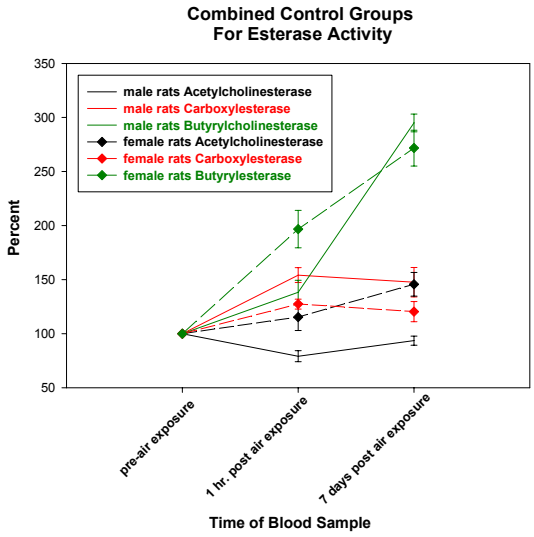

Figure 10. Effects of air exposure on rat blood esterase activity.

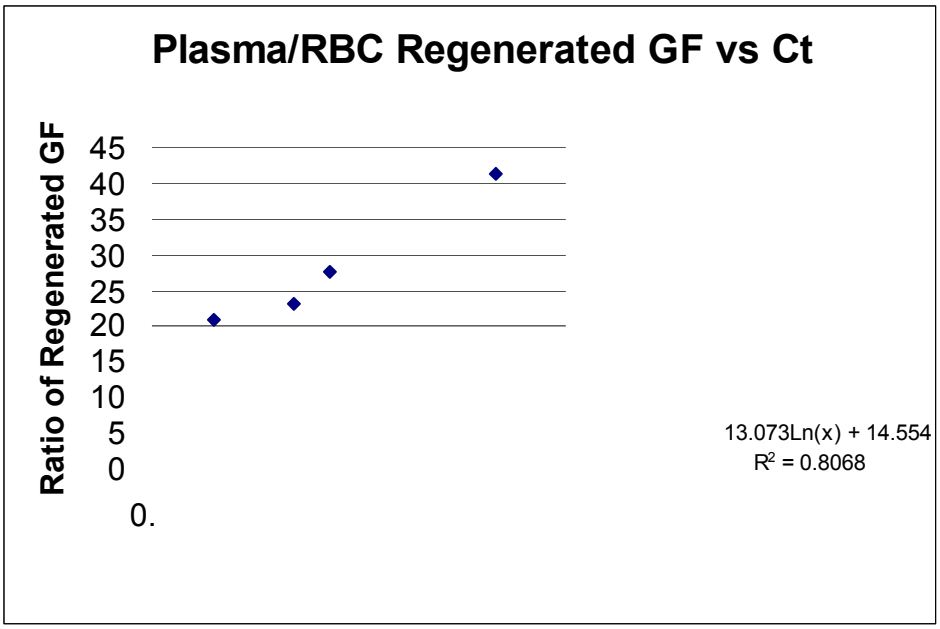

Figure 11. Regenerated GF in male and female rats after exposure.

CONCLUSIONS

Female rats are more sensitive than males to GF vapor-induced miosis. Miosis was observed in the absence of other clinical signs under exposure conditions used in this study. Depending on Ct, regenerated GF levels were 5 to 40 times higher in plasma than RBC fraction. No correlation found between miosis and circulating AChE, BuChE or CaE activity.